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ENZYMATIC SYNTHESIS OF PALM BASED FATTY MONOETHANOLAMIDE

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By

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ENZYMATIC SYNTHESIS OF PALM BASED FATTY MONOETHANOLAMIDES

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July 2002

Chairman: Professor Abu Bakar Salleh, Ph.D.

Faculty: Science and Environmental Studies

Fatty monoethanolamides, derived from a reaction between triglyceride, palm kernel olein (PKL) or free fatty acids and primary amine alcohol, monoethanolamine (MEA) can be synthesised enzymatically, under milder conditions than the energy intensive chemical reaction. Mixture of palm based fatty monoethanolamide was successfully obtained from the reaction between PKL and MEA in the presence of enzyme with better yield than the control experiment (without enzyme). The various individual fatty monoethanolamides were also obtained from the reaction between free fatty acids (C_{10}-C_{18}) and MEA after the isolation and purification processes. The various fatty monoethanolamides were used as standards for the analysis of product mixture.

Fatty monoethanolamides were characterised by infra-red spectroscopy (IR), thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) and gas chromatography

(GC). Gas chromatograph was also used for the quantitative analysis to calculate percent yield by using internal standard method.

A few types of enzyme were screened in the amidation reaction. It was found that immobilised enzymes such as Lipozyme IM, Novozyme 435, Amano PSC-lipase and AH-lipase produced better yield compared to the native enzyme, *Candida rugosa*. The results in the screening of enzyme indicated that the yield varied when different enzymes were used in the reaction mixtures. Better yield was obtained when the substrates ratio of 1:3 (PKL: MEA) was used as compared to the ratio of 1:1. It was also found that enzymes have different rates of obtaining the maximum yields. In the study, Amano PSC-lipase was observed to have good selectivity towards C_{18} fatty acid when both free fatty acid and triglyceride (PKL) were used in the amidation reaction.

In the study on the effect of temperature, the optimal yield for the PKL monoethanolamide was achieved at 60°C reaction temperature when both enzymes, Novozyme 435 and PSC-lipase were used. The yield increased ± 40-50% from the control reaction. However, the yield decreased in the presence of Novozyme 435 and PSC-lipase (14.8% and 2.6%, respectively) at temperature above 60°C, due to the temperature effect on enzyme activity. In the time course study, the optimal yield was obtained after 24h incubation time, whereas the yield for the reaction in the absence of enzyme still increased until 96 hours. The optimal ratio of substrates (PKL: MEA) was 1:5. The increase of monoethanolamine used could increase the solubility of reactants and products. In the reaction mixture, increasing the mole ratio of PKL: MEA to 1:5

increased the yield to six fold than the yield of that PKL: MEA at mole ratio 1:1. Lipase worked better in hydrophobic solvents, which have higher log P compared to hydrophilic solvents. Hexane appeared to be the best solvent for amidation reaction in the system. The reaction mixture gave the best yield when 0.1g molecular sieves were added. The yield obtained at the optimal condition in the presence of enzyme was 90%, 35% higher than that of the control experiment.

In the kinetics studies, the K_{mA} value for PSC-lipase (0.662) was slightly lower than that of Novozyme 435 (1.32) when triolein ($C_{18:1}$) was used as substrate in the reaction mixture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

SINTESIS MONOETANOLAMIDA BERLEMAK BERASASKAN MINYAK KELAPA SAWIT MENGGUNAKAN ENZIM SEBAGAI MANGKIN

Oleh

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Monoetanolamida berlemak diperolehi daripada tindakbalas antara trigliserida, fraksi olein (PKL) minyak isirong kelapa sawit atau asid lemak bebas dan alkohol amina boleh disintesis secara pemangkinan berenzim, dalam keadaan yang kurang ekstrim berbanding dengan tindakabalas kimia yang memerlukan tenaga yang intensif. Campuran monoetanolamida berlemak berasaskan minyak kelapa sawit telah berjaya dihasilkan daripada tindakbalas antara olein (PKL) minyak isirong kelapa sawit dan monoetanolamina (MEA) dengan kehadiran enzim dengan hasil yang lebih baik berbanding dengan tindakbalas kawalan (tanpa enzim). Berbagai jenis monoetanolamida berlemak tulen telah diperolehi daripada tindabalas antara asid lemak bebas (C_{10}-C_{18}) dan monoetanolamina selepas proses-proses pengasingan dan pembersihan (penulenan). Monoetanolamida-monoetanolamida berlemak tersebut digunakan sebagai piawai untuk analisis campuran produk.

Monoetanolamida berlemak telah dicirikan dengan cara spektroskopik infra merah (IR), resonans magnetik nuklear (NMR), lapisan nipis kromatografi (TLC) dan analisis kromatografi gas (GC). Kromatograf gas juga telah digunakan untuk analisis kuantitatif untuk mengira peratusan hasil dengan menggunakan kaedah piawaian dalaman.

Beberapa jenis enzim telah dikaji di dalam tindakbalas amidasi. Enzim-enzim yang telah disekat-gerak seperti Lipozyme IM, Novozyme 435, Amano PSC-lipase dan AH-lipase didapati memberikan hasil yang lebih baik berbanding dengan enzim semulajadi (asli), *Candida rugosa*. Keputusan menunjukkan hasil yang berbagai-bagai apabila enzim yang digunakan adalah berbeza di dalam campuran tindakbalas. Hasil yang lebih baik diperolehi apabila nisbah mol substrat PKL: MEA adalah 1:3 berbanding dengan 1:1. Enzim-enzim mempunyai kadar aktiviti yang berbeza untuk mencapai hasil yang optimum. Di dalam kajian perbandingan, Amano PSC-lipase diperhatikan mempunyai spesifikasi yang baik terhadap C_{18} asid lemak apabila asid lemak bebas dan trigliserida (PKL) digunakan sebagai substrat di dalam tindakbalas amidasi.

Di dalam kajian ke atas kesan suhu, hasil optima untuk campuran PKL monoethanolamida dicapai pada suhu tindakbalas 60°C apabila kedua-dua enzim, Novozyme 435 dan PSC-lipase digunakan. Hasil meningkat ± 40-50% daripada tindakbalas kawalan. Walaubagaimanapun, hasil didapati menurun dengan kehadiran Novozyme 435 dan PSC-lipase (14.8% dan 2.6%) pada suhu lebih 60°C disebabkan oleh kesan suhu ke atas aktiviti enzim. Di dalam kajian masa tindakbalas, hasil optima telah dicapai selepas 24 jam masa pengeraman, manakala hasil untuk tindakbalas tanpa enzim

terus meningkat sehingga 96 jam. Nisbah optima substrat-substrat (PKL: MEA) adalah 1:5. Monoetanolamina yang digunakan boleh meningkatkan kadar pelarutan bahan tindakbalas dan produk. Di dalam campuran tindakbalas, peningkatan nisbah mol PKL: MEA kepada 1:5 didapati meningkat hasil kepada enam kali ganda berbanding hasil daripada PKL: MEA pada nisbah mol 1:1. Lipase bertindak dengan lebih baik di dalam pelarut-pelarut hidrofobik yang mempunyai nilai log P yang lebih tinggi berbanding dengan pelarut hidrofilik. Hexana didapati sebagai pelarut terbaik untuk tindakbalas amidasi di dalam sistem tersebut. Campuran tindakbalas memberikan hasil optima apabila 0.1gram agen pengering (molecular sieve) ditambah. Hasil dicapai pada keadaan yang optimum dengan kehadiran enzim adalah 90%, iaitu 35% lebih tinggi daripada hasil dari tindakbalas kawalan.

Kajian kinetik menunjukkan nilai K_{mA} untuk PSC-lipase (0.662) adalah lebih rendah daripada Novozyme 435 (1.32) apabila triolein (C_{18}) digunakan sebagai substrat di dalam tindakbalas.

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I certify that an Examination Committee met on 27th July 2002 to conduct the final examination of Rashidah Abdul Rahim on her Doctor of Philosophy thesis entitled "Enzymatic Synthesis of Palm Based Fatty Monoethanolamides" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

RASHIDAH ABDUL RAHIM

Date: 20/8/02.

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LIST OF ABBREVIATIONS

PO	palm oil
PKO	palm kernel oil
Polein	palm olein
PS	palm stearin
PKL	palm kernel olein
PKS	palm kernel stearin
MEA	monoethanolamine
FAMEs	fatty acid methyl esters
TLC	thin layer chromatography
GC	gas chromatography
rpm	rotation perminute
NMR	nuclear magnetic resonans
IR	infrared
TMS	trimethylsilane
TMCS	trimethylchlorosilane
HMDS	hexamethyldisilazane
TNBS	trinitrobenzene sulfonate
IS	internal standard
a_w	water activity
g	gram
ml	milliliter

M	molar
µl	microliter
mg	milligram
µg	microgram
nm	nanometer
R_f	relative mobility
mmole	millimole
°C	degree Celcius
min	minute
h	hour
%	percentage
BSA	bovine serum albumin
ppm	part permillion
R_t	retention time
cm	centimetre
psi	pound persquare inch
V_{max}	maximum velocity
K_m	Michaelis-contant
K_{mA}	apparent Michaelis-contant